



Scientific &  
Educational  
Programs



1991-1992

# Joshua Lederberg

Joshua Lederberg  
President Emeritus  
University Professor  
Head of Laboratory

Visiting Professors  
Jan Sapp  
Vittorio Sgaramella

Assistant Professor  
David Thaler

Sackler Foundation Scholar  
Kenneth Zahn

Assistant for Research  
Gregory Tomblin

Adjunct Faculty  
Michiel Noordewier

This laboratory, initiated in September 1990, is focused on DNA conformation and evolutionary acceleration; namely, how modulation of the secondary and tertiary structure of DNA, and its packaging with protein complexes, influences its vulnerability to chemical alteration. As DNA conformation is already known to be a prime actor in the regulation of gene expression (e.g., supercoiling and transcriptional control), this provides a well-founded mechanism for feedback of environmental circumstance and physiological status to differential mutability of local regions of DNA. While this is contrary to current dogma ("mutations are spontaneous and evolution is directed exclusively by natural selection"), it is based on common-sense mechanistic chemistry of DNA. There are also a few dozen scattered experimental observations to support the thesis, but it has not yet been the subject of systematic critical study. We are using small *Escherichia coli* plasmids carrying several regulons that can be independently activated by external inducers. These are also associated with change in supercoiling status, and this can also be regulated in vivo and assessed in vitro. We will study how these environmentally controlled variables alter the mutational spectrum under the influence of endogenous metabolism, chemical mutagens, and transposons. We have no theoretical basis to expect any physiologically coherent regulation of the direction of mutation, but do anticipate that different sites will show differential mutability; i.e., an acceleration of evolution.

In collaboration with the Rutgers Computer Science Department, we are also setting up computer-based systems of reasoning in molecular biology, patterned on the work at Stanford on DENDRAL during the 1970s. This logical reconstruction is expected to be of great assistance in experiment planning, and in the organization and retrieval of the vast amount of information recorded in the published literature.

## PUBLICATIONS

- Lederberg, J. 1987. Genetic recombination in bacteria: a discovery account. *Annual Review of Genetics*. 21:23–46.
- Lederberg, J. 1988. Pandemic as a natural evolutionary phenomenon. *Social Research*. 55:342–357.
- Lederberg, J. 1988. The ontogeny of the clonal selection theory of antibody formation: reflections on Darwin and Ehrlich. *Annals of the New York Academy of Sciences*. 546:175–187.
- Lederberg, J. 1989. Replica plating and indirect selection of bacterial mutants: isolation of preadaptive mutants in bacteria by sib selection. *Genetics*. 121:395–399.
- Lederberg, J. 1990. How DENDRAL was conceived and born. In *A History of Medical Informatics*. B. I. Blum and K. Duncan, editors. New York: ACM Press & Addison Wesley, pp. 14–14.
- Lederberg, J. 1990. Excitement and Fascination of Science. Volume III, Parts 1 and 2. Palo Alto, CA: Annual Reviews, Inc., part.1 1297 pp. + index; part 2, 1301–2338.
- Rayssiguier, C., M. Radman, and D. S. Thaler. 1989. The barrier to recombination between *Escherichia coli* and *Salmonella typhimurium* is disrupted in mismatch-repair mutants. *Nature*. 342:396–401.
- Russell, C. B., D. S. Thaler, and F. W. Dahlquist. 1989. Chromosomal transformation of *Escherichia coli* RecD strains with linearized plasmids. *Journal of Bacteriology*. 171:2609–2613.
- Schnos, M., F. R. Blattner, R. B. Inman, and K. Zahn. 1989. DNA looping induced by bacteriophage- $\lambda$  O-Protein: implications for formation of higher-order structures at the  $\lambda$ -origin of replication. *Virology*. 168:370–377.
- Searls, D. B., and M. O. Noordewier. 1991. Pattern-matching search of DNA sequences using logic grammars. IEEE Computer Society. *Proceedings of the Annual Conference on Artificial Intelligence Applications*. 7, 3–10.
- Thaler, D. S., and F. W. Stahl. 1988. DNA double-chain breaks in recombination of phage- $\lambda$  and of yeast. *Annual Review of Genetics*. 22:169–197.
- Thaler, D. S., L. C. Thomason, I. Siddiqi, M. M. Stahl, F. W. Stahl, S. M. Rosenberg, and E. Sampson. 1989. Recombination of bacteriophage- $\lambda$  in RecD mutants of *Escherichia coli*. *Genome*. 31:53–67.

# *The Rockefeller University*

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Our laboratory is focused on DNA conformation and evolutionary acceleration, i.e., how modulation of the secondary and tertiary structure of DNA, and its packaging with protein complexes, influences its vulnerability to chemical alteration. As DNA conformation is already known to be a prime actor in the regulation of gene expression (e.g., supercoiling and transcriptional control), this provides a well-founded mechanism for feedback of environmental circumstance and physiological status to differential mutability of local regions of DNA. While this is contrary to current dogma ("mutations are spontaneous and evolution is directed exclusively by natural selection"), it is based on common-sense mechanistic chemistry of DNA. There are also a few dozen scattered experimental observations to support the thesis, but it has not yet been the subject of systematic critical study. We are using small *Escherichia coli* plasmids carrying several regulons that can be independently activated by external inducers. These are also associated with change in supercoiling status, and this can also be regulated in vivo and assessed in vitro. We are studying how these environmentally controlled variables alter the mutational spectrum under the influence of endogenous metabolism, chemical mutagens, and transposons. To this end, we have developed approaches to the fingerprinting of mutant sequences with PCR-related technology. In collaboration with the Rutgers Computer Science Department, we are also setting up computer-based systems of reasoning in molecular biology, patterned on the work at Stanford on DENDRAL during the 1970s. This logical reconstruction is expected to be of great assistance in experiment planning, and in the organization and retrieval of the vast amount of information recorded in the published literature.

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- Lederberg, J. 1992. The anti-expert system: hypotheses an AI program should have seen through. In *Artificial Intelligence and Molecular Biology*. L. Hunter, editor. Menlo Park, CA: AAAI Press. In press.
- Lederberg, J. 1992. Bacterial variation since Pasteur. *ASM News*. 58:261-265.
- Le Dérout, J., D. S. Thaler, N. Guillén, and L. Hirschbein. 1992. The *spoOA* gene is implicated in the maintenance of noncomplementing diploids in *Bacillus subtilis*. *Molecular Microbiology*. 6:1495-1505.
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Scientific and Educational Programs



1993-1994

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### DAVID THALER

I study the molecular mechanisms with which living things change in a heritable way: mutation, recombination, and imprinting. The template chain of a transcribed gene transiently participates in a hybrid with the message. RNA-DNA hybrids are also present as intermediates in replication and reverse transcription. We (myself in collaboration with Kenneth Zahn and Greg Tomblin) are studying the genetics (mutation and recombination) of these hybrids. Consideration of polynucleotide hybrids began as a hypothesis for a mechanism of endogenous mutagenesis; however, this work also has relevance in the context of antisense pharmacology.

recA and recD are important genes for recombination and mutation in *E. coli*. Julie Losman and I are developing new assays for recA's binding to damaged sites on DNA. Joe Heitman, John Huang, Grace Chen, and I have found that recD mutants are remarkably resistant to some types of DNA damage. At the moment I'm trying to figure out why *E. coli* should bother to keep around a gene that makes it more sensitive to, for example, gamma rays.

C. Rayssiguier, M. Radman, and I have found that the amount of sequence similarity required for genetic recombination is itself under genetic control. Some implications of this finding are being considered in collaboration with Michiel Noordewier and Joshua Lederberg. We've developed methods to quantitate the similarity tolerance required to maintain the integrity of a genome. We're working towards algorithms for similarity searches derived from biological parameters, i.e. characteristics which determine whether sequences recombine in vivo.

KENNETH ZAHN

My area of research concerns the formation and detection of alternative structures in DNA with the goal of development of nondestructive methods of detection of mutational change in DNA. The method involves the generation of RNA copies of regions of difference between two DNA molecules through the property of nonspecific transcription of single-stranded DNA by T7 RNA polymerase. The initial analysis has consisted of characterization of the mode and products of transcription by T7 RNA polymerase on single-stranded DNA and artificial heteroduplexes.

Additional areas of interest concern various aspects of bacteriophage lambda biology, especially (*a*) the nature of the initial structural transitions during melting of the origin of replication by the phage *ori* recognition protein and (*b*) control of the integration of lambda DNA into the *E. coli* chromosome through translational regulation of the phage integration protein. Toward these ends, variants of phage lambda are being constructed with altered replication origins and integrase genes for testing *in vivo*.

SRINIVAS SASTRY

We are interested in the interactions of procaryotic DNA-dependent RNA polymerases with DNA templates and nascent RNA transcripts. We are studying transcriptional elongation as a regulatory process in relation to DNA replication and DNA-protein cross-link repair. Transcriptional elongation by *E. coli* and phage T7 RNA polymerases was arrested by specifically placing a psoralen drug molecule downstream of a promoter. We are studying the structure of the arrested ternary complex. Specific laser-induced cross-linking of the arrested RNA polymerase to the DNA template was used to prepare highly stable elongation complexes. Proteolytic digestion and high performance liquid chromatography purification methods are used to isolate peptides of RNA polymerase conjugated to psoralen and DNA. The microscopic contacts between the amino acid side chains and DNA bases via psoralen are mapped by amino acid sequence analysis, peptide sequencing, and mass spectrometry. We are constructing model plasmids to understand DNA topology and interactions between DNA polymerases and RNA polymerases during replication and transcription. Repair of site-specific DNA-protein cross-links is also being investigated.

#### MICHIEL NOORDEWIER

We are proceeding along two directions at the interface of computer science and molecular biology: (1) the development and testing of computational systems for searching and analyzing biological sequences, and (2) the design of a knowledge-based system for theory refinement in molecular biology.

Computational tools for analyzing sequences include the use of information theory for the characterization of control signals in gene sequences (with Martin Farach of Rutgers University, and Larry Shepp and Jakob Ziv of AT&T Bell Laboratories). In addition, with Haym Hirsh (Rutgers University) and Craig Benham (Mount Sinai Medical School), we are using background knowledge of the physical and chemical properties of DNA to improve inductive learning of rules for genetic sequences. With David Axelrod (Rutgers University), we are using these tools to develop a characterization of the large-scale structure of the eukaryotic genome.

With Michael Cook, we have developed an enhanced string language for representing nucleic acids. It includes conventions for representing single-stranded molecules, double-stranded molecules, RNA, DNA, and RNA-DNA hybrids; for distinguishing between the two strands of a double-stranded molecule; and for keeping track of the 5' to 3' orientation of a sequence. Our formalism supports operations representing the action of basic enzymes used in genetic engineering. We have used this formalism to prototype a rule-based system in PROLOG which interprets the results of gel electrophoresis experiments in molecular genetics.

#### PUBLICATIONS

Lederberg, J., editor-in-chief. 1992. *Encyclopedia of Microbiology*. 4 volumes. San Diego: Academic Press, Inc.

Lederberg, J., R. E. Shope, and S. C. Oaks, editors. 1992. *Emerging Infections: Microbial Threats to Health in the United States*. Washington, DC: National Academy Press. 294 pp.

Sastry, S. S., and J. E. Hearst. 1991. Studies on the interaction of T<sup>7</sup> RNA polymerase with a DNA template containing a site-specifically placed Psoralen cross-link. I. Characterization of elongation complexes. II. Stability and some properties of elongation complexes. *Journal of Molecular Biology* 221:1091-1110; 1111-1125.

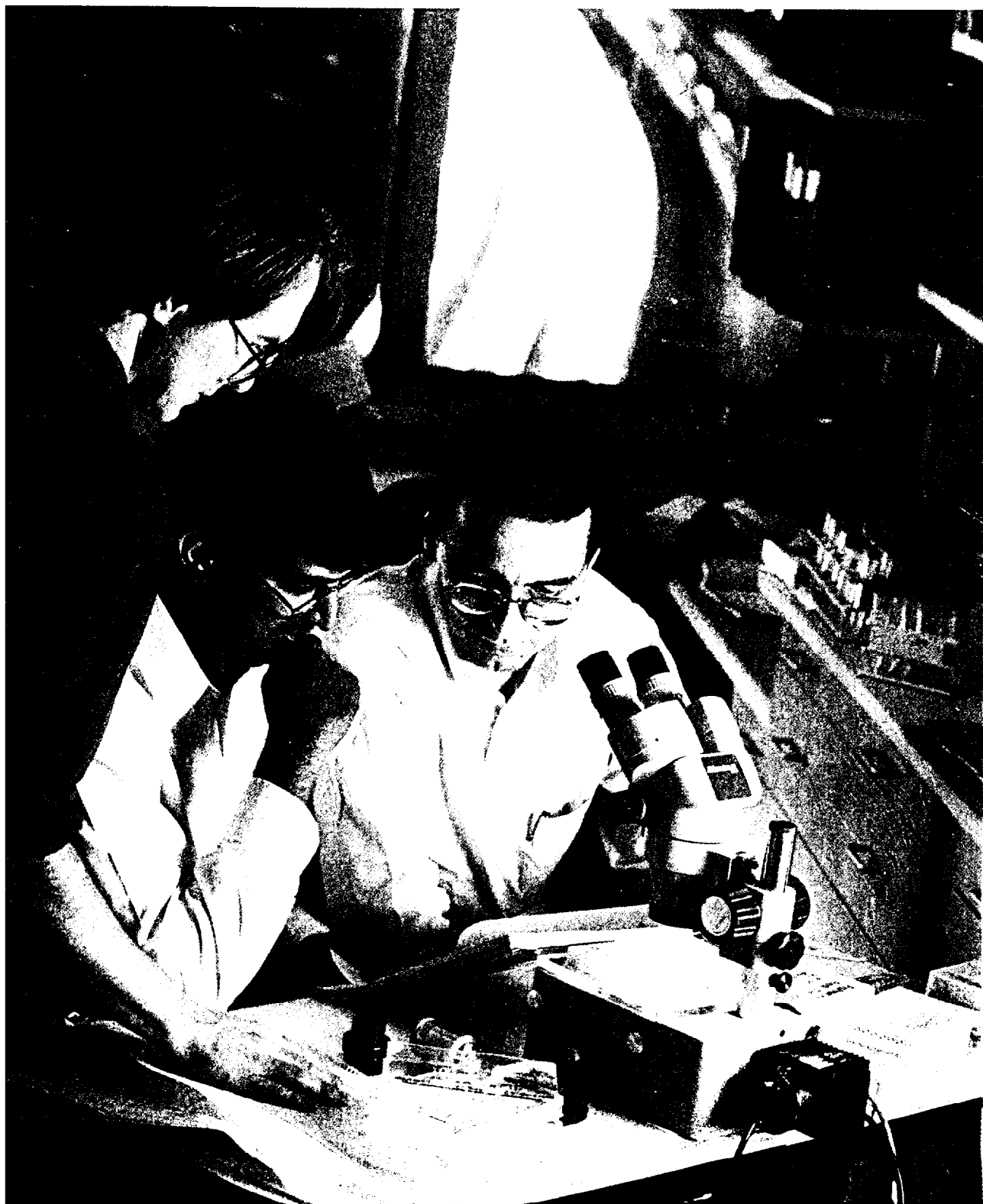
Sastry, S. S., S. S. Spielmann, Q. S. Hoang, A. M. Phillips, A. Sancar, and J. E. Hearst. 1993. Laser-induced protein-DNA cross-links via psoralen furanside monoadducts. *Biochemistry*. In press.

Spielmann, P. H., S. S. Sastry, and J. E. Hearst. 1992. New methods for the large-scale preparation of psoralen furanside monoadducts and diadducts. *Proceedings of the National Academy of Sciences* 89:4514-4518.

Thaler, D. S., J. R. Roth, and L. Hirschbein. 1990. Imprinting as a mechanism for the control of gene expression. In *The Bacterial Chromosome*. K. Drlica and M. Riley, editors. Washington, DC: American Society of Microbiology. pp. 445-456.

# The Rockefeller University

Scientific and Educational Programs 1994 - 1995



Annual Report 1993-1994

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### STEPHEN S. MORSE

*Viral Infections of T Lymphocytes: Pathogenesis and Evolution.* The major goal of this laboratory is a better understanding of how viruses interact with the host immune system, especially T lymphocytes, to cause disease. Most of our work involves mouse thymic virus (MTLV, a herpesvirus) and human immunodeficiency virus (HIV, a lentivirus).

MTLV kills CD4<sup>+</sup> T lymphocytes in the neo-natal mouse thymus. This cell death may result from clonal deletion, a host mechanism which normally eliminates self-reactive T lymphocytes. In MTLV-infected mice, the thymus eventually regenerates but a number of the mice develop autoantibodies later in life. We are continuing to concentrate on the relationship between thymic necrosis after infection, clonal deletion mechanisms, and autoimmunity.

MTLV shows biological similarities to two recently discovered human herpesviruses, HHV 6 and 7, which also infect T lymphocytes. Our preliminary work indicates apparent antigenic cross-reactivity and DNA cross-hybridization between MTLV and HHV-6. We have been developing a polymerase chain reaction detection system for MTLV, in collaboration with Pamela B. Moore (Director of the LARC Diagnostic Laboratory), in order to further compare pathogenesis and DNA homologies.

Collaborating with colleagues at Cornell University Medical College, we are defining thymic involvement in pediatric HIV infection. Circulating CD4<sup>+</sup>8<sup>+</sup> T cells, immature cells normally found only in the thymus, have been identified in a number of HIV-infected children. We are further characterizing the developmental stage of these cells. Using similar approaches, we are comparing immunopathogenesis of feline immunodeficiency virus, a lentivirus that shows promise as a laboratory animal model for AIDS.

## PUBLICATIONS

- Morse, S. S. 1990. Comparative sensitivity of infectivity assay and mouse antibody production (MAP) test for detection of mouse thymic virus (MTLV). *Journal of Virological Methods*. 28:15-24.
- Morse, S. S. 1990. Looking for a link (interspecies transfer of viruses). *Nature*. 344:297.
- Morse, S. S. 1991. Emerging viruses: defining the rules for viral traffic. *Perspectives in Biology and Medicine*. 34:387-409.
- Morse, S. S. 1992. What do we know about the origins of emerging viruses? In *Emerging Viruses*. S. S. Morse, editor. New York and Oxford: Oxford University Press. In press.
- Morse, S. S. 1992. "Emerging Viruses" and "Viral Evolution." In *Encyclopedia of Microbiology*. J. Lederberg, editor. Orlando, FL: Academic Press, Inc. In press.
- Morse, S. S., and A. Schluederberg. 1990. Emerging viruses: the evolution of viruses and viral diseases. *Journal of Infectious Diseases*. 162:1-7.

## SRINIVAS SASTRY

We are studying mechanisms of transcriptional initiation and elongation. Our goal is to understand the conformational transitions in the RNA polymerase molecule as it moves down the DNA template. We are attempting to map the DNA-contacting amino acid side chains in the RNA polymerase by photochemical cross-linking approaches. Transcriptional initiation and elongation complexes of *E. coli* and phage T7 RNA polymerases are prepared by arresting the polymerase at specific sites on the DNA templates. Specific cross-linking of the arrested RNA polymerase to the DNA template is induced by high intensity 365-nm UV light, and a site-specific photochemical probe on the DNA template. Highly stable transcription initiation and elongation complexes are purified by high performance liquid chromatography. The molecular contacts between the amino acid side chains in the RNA polymerase and the DNA bases are mapped by a variety of techniques, including proteolytic degradation of the polymerase followed by mass spectrometry, amino acid composition analysis, and peptide sequence analysis.

My hypothesis is that protein-DNA cross-links are a major source of DNA damage from solar UV, and that protein-DNA cross-links are formed at 1-2 orders of magnitude greater than the estimated number of DNA interstrand cross-links (~8/cell/day). To understand the repair of DNA-protein cross-links in vitro and in vivo, we are constructing model systems that have zero-length and bulky adduct-mediated protein-DNA cross-links.

## DAVID THALER

*Is RNA a Mutagen?* RNA-DNA hybrids are essential at several key junctures in biology as: (a) intermediates in transcription; (b) primers for DNA replication at the origin and throughout the chromosome on the lagging strand; (c) primers for reverse transcription in retroviruses. Our work on RNA-DNA hybrids is motivated by the hypothesis that the location and types of genetic change (mutation and recombination) engendered could be targeted and physiologically modulated via hybrids of RNA and DNA. Model substrates are used to study the genetic properties of RNA-DNA hybrids both in vivo and in vitro.

We've found that information on RNA whose phosphodiester backbone is covalently continuous with DNA can be inherited in vivo in wild type *E. coli*. Information transfer from RNA to DNA could occur via either mismatch correction or an activity of DNA polymerase that allows it to cross a covalently continuous backbone between DNA and RNA. Tests of

the polymerase hypothesis in vitro indicate that several DNA polymerases can become cis-acting reverse transcriptases, if, and only if, they get a "running start" on a DNA backbone.

*Extending the Biochemical Specificity Required for Genetic Information Transfer.*

DNA was the first chemical shown capable of carrying genes. Some years later it was found that RNA can also carry genetic information and our work extends the context in which RNA can so act. Modifying the backbone structure of polynucleotides in other ways allows us to assay novel chemistries for their ability to transfer genetic information. So far we have found that several decorations of DNA and RNA with methyl and sulfur are quite "alive" in the genetic sense. This work has implications for antisense pharmacologies that use modified backbones.

"We" in this section refers to Shumo Liu, Julie Losman, José Reyes, David Thaler, Greg Tomblin, and Ken Zahn.

#### KENNETH ZAHN

One major area of my work is the development of molecular tools for the detection of mutational change in DNA molecules. The method I am developing is nondestructive in that it generates RNA copies of regions of difference in heteroduplex DNA. It uses the property of nonspecific recognition and transcription initiation on heteroduplex "bubbles" by T7 RNA polymerase. As such, it bypasses the need for a conventional promoter. Initial analyses on artificial heteroduplexes demonstrate that insertion/deletion heteroduplexes serve as unidirectional promoters and substitution heteroduplexes as bidirectional promoters. The transcription products made from such DNA substrates are currently under close scrutiny. It is hoped that such a method may be applied to larger genome analysis.

Additional areas of interest are aspects of bacteriophage lambda biology, especially (a) DNA protein interactions involved in the recognition of the replication origin by the initiator protein and the nature of the structural transition in supercoiled DNA caused by this interaction. Toward these ends, genetic systems are currently being developed, using gene and protein fusions such that replication is no longer the assay for ori or ori-recognition protein function. (b) Control of the integration of lambda DNA into the *E. coli* chromosome through translational regulation of the phage integration protein. In this regard protein fusions of integrase to  $\beta$ -galactosidase under the control of different promoters are being used to quantitate the influence of the intragenic and extragenic regulatory sequences in question. In addition, small plasmid molecules containing the integrase gene controlled by its natural promoter (pI) and positive activator (cII protein) and others containing a transfer RNA gene have been constructed to demonstrate the regulatory effects of specific tRNAs within a context which resembles that occurring within the cell during viral infection.

#### MICHIEL NOORDEWIER

My current research efforts are proceeding along two directions at the interface of computer science and molecular biology. The efforts are aimed at the development and testing of computational systems for searching and analyzing biological sequences.

Computational tools for analyzing sequences include the use of information theory for the characterization of control signals in gene sequences (with Martin Farach of Rutgers University, and Larry Shepp and Jakob Ziv of AT&T Bell Laboratories). In addition, with Haym Hirsh (Rutgers University) and Craig Benham (Mount Sinai School of Medicine), we are using background knowledge of the physical and chemical properties of DNA to improve inductive learning of rules for genetic sequences.

With Sumit Ganguly (Rutgers University) we are developing an index structure and retrieval system for biological sequence databases which promises to be efficient and general. This organization of the databases will complement other current efforts at sequence comparison and analysis, by providing an infrastructure in which other methods can be used to efficiently locate desired sequences.

Finally, with Martin Farach and David Axelrod (Rutgers University) we are using results from the field of text data compression to create faster methods for screening large databases for the nonrandom structures in DNA. The discovery of genomic structure is equivalent to the discovery of repeats of sequences or their transforms. These repeated structures are abundant in higher organisms, but their biological and structural significance is not fully understood. There are hints that they are important for gene regulation, evolution, genome macrostructure, chromosome function, human disease, and protein function and processing. We are developing tools to characterize such structure in eukaryotic genomes. These tools will allow biologists to phrase sequence analysis tasks in abstract terms, without a need to implement or alter string-level comparison tasks.

#### PUBLICATIONS

Hirsh, H., and M. Noordewier. 1994. Using background knowledge to improve inductive learning of DNA sequences. *IEEE (Institute of Electrical and Electronics Engineers) Expert*. In press.

Lederberg, J. 1993. Emerging infections: microbial threats to health. *Trends in Microbiology*. 1:43-44.

Lederberg, J. 1994. The transformation of genetics by DNA: an anniversary celebration of Avery, MacLeod, and McCarty. *Genetics*. 136:423-426.

Le Derout, J., D. S. Thaler, et al. 1992. The spoOA gene is implicated in the maintenance of non-complementing diploids in *Bacillus subtilis*. *Molecular Microbiology*. 6:1495-1505.

Lindsay, R. K., B. B. Buchanan, E. A. Feigenbaum, and J. Lederberg. 1993. Dendral: a case study of the first expert system for scientific hypothesis formation. *Artificial Intelligence*. 61: 209-261.

Rayssiguier, C., D. S. Thaler, et al. 1989. The barrier to recombination between *Escherichia coli* and *Salmonella typhimurium* is disrupted in mismatch-repair mutants. *Nature*. 342:396-401.

Reardon, J. T., P. H. Spielmann, J.-C. Huang, S. S. Sastry, A. Sancar, and J. E. Hearst. 1991. Removal of psoralen monoadducts and crosslinks by human cell-free extracts. *Nucleic Acids Research*. 17:4623-4629.

Russell, C. B., D. S. Thaler, et al. 1989. Chromosomal transformation of *Escherichia coli* recD strains with linearized plasmids. *Journal of Bacteriology*. 171:2609-2613.

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Sastry, S. S., H. P. Spielmann, and J. E. Hearst. 1992. Psoralens and their application to the study of some molecular biological processes. *Advances in Enzymology*. 66:85-148.

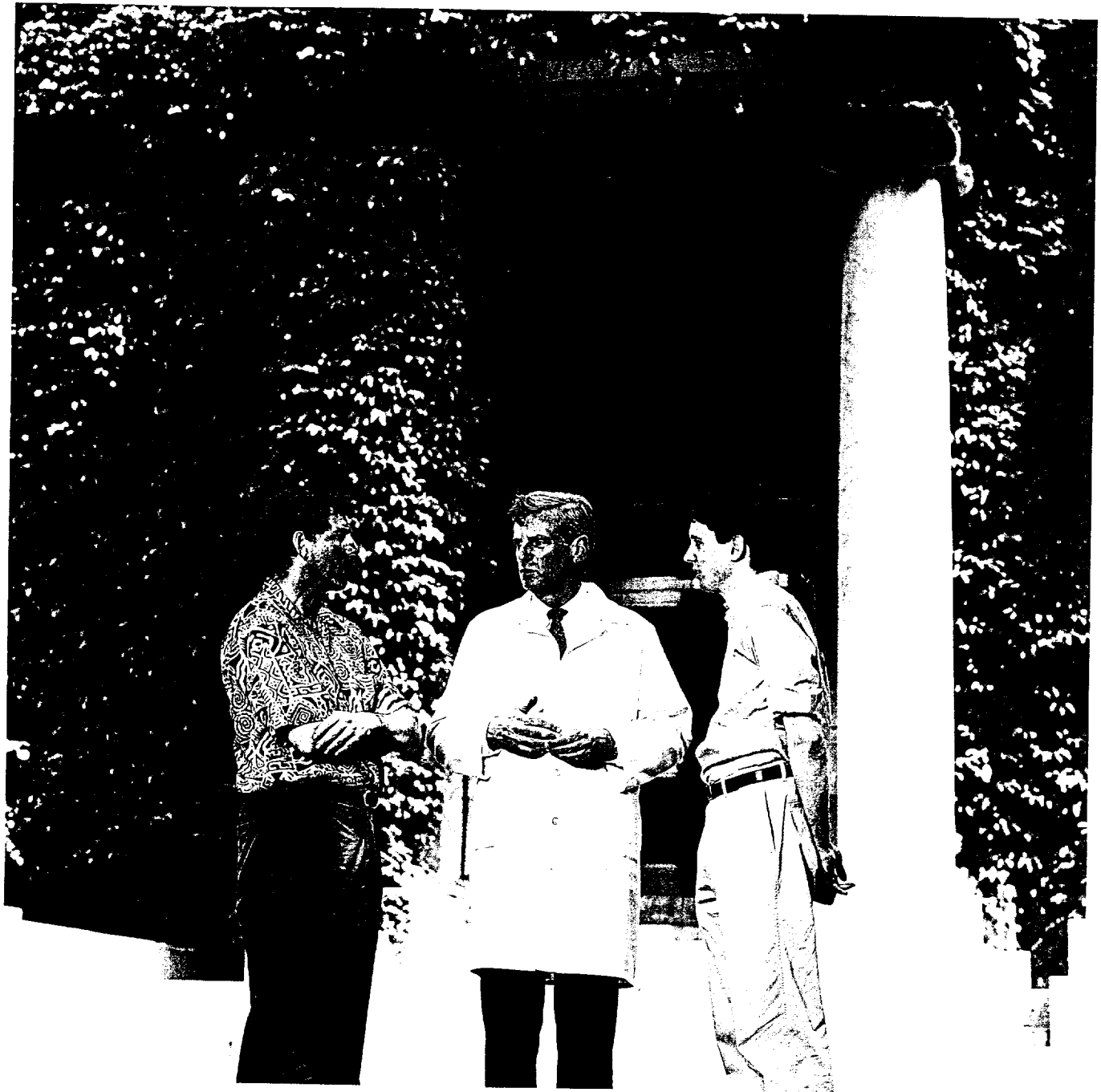
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Thaler, D. S. 1994. The evolution of genetic intelligence. 1994. *Science*. In press.

Thaler, D. S. 1994. Sex is for sisters: intragenomic recombination and homology-dependent mutation as sources of evolutionary variation. *Trends in Ecology and Evolution*. 9:108-110.

- Thaler, D. S., and M. O. Noordewier. 1992. MEPS parameters and graph analysis for the use of recombination to construct ordered sets of overlapping clones. *Genomics*. 13:1065–1074.
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- Thaler, D. S., and F. W. Stahl. 1988. DNA double-chain breaks in recombination of phage lambda and of yeast. *Annual Review of Genetics*. 22:169–197.
- Wolff, J. A., and J. Lederberg. 1994. A history of gene transfer and therapy. *Human Gene Therapy*. 5:469–480.

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*1994–1995 Annual Report*

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President Emeritus  
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Raymond and Beverly Sackler  
Foundation Scholar

### Assistant Professors

Srinivas Sastry  
David S. Thaler

### Senior Research Associate

Pamela B. Moore

### Guest Investigators

Wei Chen  
Kenneth C. Zahn

### Graduate Fellow

Bradley Messmer

### Assistants for Research

Shumo Liu  
Barbara M. Ross

### Adjunct Faculty

Lindley Darden  
Edward Hackett  
Robert K. Merton  
Michiel O. Noordewier  
Harriet Zuckerman

### Also associated with the laboratory:

#### Assistant Professor

Stephen S. Morse

#### Postdoctoral Fellow

Karen M. Manchester

#### Assistants for Research

James J. Sullivan  
Stephen S. To

#### Adjunct Faculty

Toby C. Rodman

Our laboratory is focused on DNA conformation and evolutionary acceleration; namely, how modulation of the secondary and tertiary structure of DNA, and its packaging with protein complexes, influences its vulnerability to chemical alteration. As DNA conformation is already known to be a prime actor in the regulation of gene expression (e.g., supercoiling and transcriptional control), this provides a well-founded mechanism for feedback of environmental circumstances and physiological status to differential mutability of local regions of DNA. A few dozen scattered experimental observations support the thesis, but it has not yet been the subject of systematic critical study. We are using small *Escherichia coli* plasmids carrying regulons that can be independently activated by external inducers. These are also associated with change in supercoiling status, and this can also be regulated *in vivo* and assessed *in vitro*. We are studying how these environmentally controlled variables alter the mutational spectrum consequent to the influence of endogenous metabolism, chemical mutagens, and transposons. In collaboration with the Rutgers Computer Science Department, we are also setting up computer-based systems of reasoning in molecular biology, patterned on the work at Stanford on DENDRAL during the 1970s. This logical reconstruction is expected to be of great assistance in experiment planning, and in the organization and retrieval of the vast amount of information recorded in the published literature.

### Michiel Noordewier

Mick Noordewier's interests are aimed at the development and testing of computational systems for searching and analyzing biological sequences. Our research efforts have recently proceeded along four major directions:

1. With Haym Hirsh (Rutgers University) and Craig Benham (Mt. Sinai Medical School), we are utilizing background knowledge of the physical and chemical properties of DNA to improve inductive learning of rules for genetic sequences.
2. With Sumit Ganguly (Rutgers University) we are developing an index structure and retrieval system for biological sequence databases which promises to be efficient and general.
3. We have explored the use of information theory for the characterization of control signals in gene sequences (with Martin Farach of Rutgers University, and Larry Shepp and Jakob Ziv of AT&T Bell Laboratories).
4. Finally, with Martin Farach and David Axelrod (Rutgers University), we are developing tools to characterize such structure in eukaryotic genomes. These tools will allow biologists to phrase sequence analysis tasks in abstract terms, without a need to implement or alter string-level comparison tasks.

### David Thaler

*Is RNA a Mutagen?* RNA-DNA hybrids are essential at several key junctures in biology as: (a) intermediates in transcription; (b) primers for DNA replication at the origin and throughout the chromosome on the lagging strand; (c) primers for reverse transcription in retroviruses. Our work on RNA-DNA hybrids is motivated by the hypothesis that the location and types of genetic change (mutation and recombination) engendered could be targeted and physiologically modulated via hybrids of RNA and DNA. Model substrates

are used to study the genetic properties of RNA–DNA hybrids both *in vivo* and *in vitro*.

We've found that information on RNA whose phosphodiester backbone is covalently continuous with DNA can be inherited *in vivo* in wild type *E. coli*. Information transfer from RNA to DNA could occur via either mismatch correction or an activity of DNA polymerase that allows it to cross a covalently continuous backbone between DNA and RNA. Tests of the polymerase hypothesis *in vitro* indicate that several DNA polymerases can become cis-acting reverse transcriptases, if, and only if, they get a "running start" on a DNA backbone.

*Extending the Biochemical Specificity Required for Genetic Information Transfer.* DNA was the first chemical shown capable of carrying genes. Some years later it was found that RNA can also carry genetic information and our work extends the context in which RNA can so act. Modifying the backbone structure of polynucleotides in other ways allows us to assay novel chemistries for their ability to transfer genetic information. So far we have found that several decorations of DNA and RNA with methyl and sulfur are quite "alive" in the genetic sense. This work has implications for antisense pharmacologies that use modified backbones.

"We" in this section refers to Shumo Liu, Bradley Messmer, José Reyes, David Thaler, Greg Tomblin, and Ken Zahn.

### Srinivas Sastry

We are studying mechanisms of transcriptional initiation and elongation. Our goal is to understand the conformational transitions in the RNA polymerase molecule as it moves down the DNA template. We are attempting to map the DNA-contacting amino acid side chains in the RNA polymerase by photochemical cross-linking approaches. Transcriptional initiation and elongation complexes of *E. coli* and phage T7 RNA polymerases are prepared by arresting the polymerase at specific sites on the DNA templates. Specific cross linking of the arrested RNA polymerase to the DNA template is induced by high intensity 365-nm UV light, and a site-specific photochemical probe on the DNA template. Highly stable transcription initiation and elongation complexes are purified by high performance liquid chromatography. The molecular contacts between the amino acid side chains in the RNA polymerase and the DNA bases are mapped by a variety of techniques, including proteolytic degradation of the polymerase followed by mass spectrometry, amino acid composition analysis, and peptide sequence analysis.

My hypothesis is that protein–DNA cross-links are a major source of DNA damage from solar UV, and that protein–DNA cross-links are formed at 1–2 orders of magnitude greater than the estimated number of DNA interstrand cross-links (~8/cell/day). To understand the repair of DNA–protein cross-links *in vitro* and *in vivo*, we are constructing model systems that have zero length and bulky adduct-mediated protein–DNA cross-links.

### PUBLICATIONS

Farach, M., M. Noordewier, S. Savari, L. Shepp, A. Wyner and J. Ziv. 1994. On the entropy of DNA: algorithms and measurements based on memory and rapid convergence. *Symposium on Discrete Algorithms, San Francisco*

Ganguly, S., and M. Noordewier. 1995. A database for the efficient retrieval of biological sequences. *Computers in Biology and Medicine*. In press

- Hirsh, H., and M. Noordewier. 1994. Using background knowledge to improve inductive learning. *IEEE Expert*. 9(5):3–6.
- Lederberg, J. 1993. Emerging infections: microbial threats to health. *Trends in Microbiology*. 1:43–44.
- Lederberg, J. 1994. The transformation of genetics by DNA: an anniversary celebration of Avery, MacLeod, and McCarty. *Genetics*. 136:423–426.
- LeDerout, J., D. S. Thaler, *et al.* 1992. The *spoOA* gene is implicated in the maintenance of non-complementing diploids in *Bacillus subtilis*. *Molecular Microbiology*. 6:1495–1505.
- Lindsay, R. K., B. B. Buchanan, E. A. Feigenbaum, and J. Lederberg. 1993. Dendral: a case study of the first expert system for scientific hypothesis formation. *Artificial Intelligence*. 61:209–261.
- Rayssiguier, C., D. S. Thaler, *et al.* 1989. The barrier to recombination between *Escherichia coli* and *Salmonella typhimurium* is disrupted in mismatch-repair mutants. *Nature*. 342:396–401.
- Reardon, J. T., P. H. Spielmann, J.-C. Huang, S. S. Sastry, A. Sancar, and J. E. Hearst. 1991. Removal of psoralen monoadducts and crosslinks by human cell-free extracts. *Nucleic Acids Research*. 17:4623–4629.
- Russell, C. B., D. S. Thaler, *et al.* 1989. Chromosomal transformation of *Escherichia coli* recD strains with linearized plasmids. *Journal of Bacteriology*. 171:2609–2613.
- Sastry, S. S., and J. E. Hearst. 1991. Studies on the interaction of T7 RNA polymerase with a DNA template containing a site-specifically placed psoralen cross-link. I. Characterization of elongation complexes. *Journal of Molecular Biology*. 221:1091–1110.
- Sastry, S. S., and J. E. Hearst. 1991. Studies on the interaction of T7 RNA polymerase with a DNA template containing a site-specifically placed psoralen cross-link. II. Stability and some properties of elongation complexes. *Journal of Molecular Biology*. 221:1111–1125.
- Sastry, S. S., H. P. Spielmann, and J. E. Hearst. 1992. Psoralens and their application to the study of some molecular biological processes. *Advances in Enzymology*. 65:85–148.
- Sastry, S. S., H. P. Spielmann, D. S. Hoang, A. M. Phillips, A. Sancar, and J. E. Hearst. 1993. Laser-induced protein–DNA cross-links via psoralen furanside monoadducts. *Biochemistry*. 32:5526–5538.
- Sastry, S. S., H. P. Spielmann, T. J. Dwyer, D. E. Wemmer, and J. E. Hearst. 1992. Recent advances in the synthesis and structure determination of site specifically psoralen modified DNA oligonucleotides. *Journal of Photochemistry and Photobiology B Biology*. 14:65–79.
- Sastry, S., and P. L. Hoffman. 1995. The influence of RNA and DNA template structures during transcript elongation by RNA polymerases. *Biochemical and Biophysical Research Communications*. 211:106–114.
- Spielmann, H. P., T. J. Dwyer, S. S. Sastry, J. E. Hearst, and D. E. Wemmer. 1995. DNA conformational reorganization upon conversion of a psoralen furanside monoadduct to an interstrand cross-link: Implications for DNA repair. *Proceedings of the National Academy of Sciences USA*. 92:2345–2349.
- Thaler, D. S. 1994. Sex is for sisters: intragenomic recombination and homology-dependent mutation as sources of evolutionary variation. *Trends in Ecology and Evolution*. 9:109–110.
- Thaler, D. S. 1994. The evolution of genetic intelligence. *Science*. 264:224–225.
- Thaler, D. S., and B. T. Messmer. 1995. Evolution of genetic intelligence. In *The Encyclopedia of Molecular Biology: Fundamentals and Applications*.
- Thaler, D. S., and F. W. Stahl. 1988. DNA double-chain breaks in recombination of phage lambda and of yeast. *Annual Review of Genetics*. 22:169–197.
- Thaler, D. S., and M. O. Noordewier. 1992. MEPS parameters and graph analysis for the use of recombination to construct ordered sets of overlapping clones. *Genomics*. 13:1065–1074.
- Thaler, D. S., G. Tomblin, and K. Zahn. 1995. Short-patch reverse transcription in *Escherichia coli*. *Genetics*. 140:909–915.
- Thaler, D. S., J. R. Roth, *et al.* 1990. Imprinting as a mechanism for the control of gene expression. In *The Bacterial Chromosome*. K. Orlica and M. Riley, editors. American Society of Microbiology: Washington, D.C. pp. 445–456.
- Thaler, D. S., S. Liu, and G. Tomblin. 1995. Extending the chemistry that supports genetic information transfer *in vivo*: phosphorothioate DNA, phosphorothioate RNA, 2'-O-methyl RNA and methylphosphorothioate DNA. In press.
- Wolff, J. A., and J. Lederberg. 1994. A history of gene transfer and therapy. *Human Gene Therapy*. 5:469–480.

**Stephen S. Morse**

Our major focus is in viral pathogenesis, especially in the immune system, and models for studying viral diseases. A related focus is in molecular evolution of viruses, and in factors responsible for viral emergence.

*CD4 Cell Depletion by Viruses.* Mouse thymic virus (MTLV) is a herpesvirus that infects and kills developing T lymphocytes in newborn mice, although the virus probably preferentially infects T cells in mice of any age. In the neonatal mouse thymus, and in other lymphoid tissues such as spleen, MTLV infection selectively kills CD<sup>4+</sup> T lymphocytes. Our recent evidence suggests that apoptosis (programmed cell death) is induced in the lymphocytes during infection and appears to be responsible for this cell depletion. We are currently investigating how apoptosis may be triggered during infection, including possible involvement of superantigens and thymic mechanisms for negative selection.

Despite massive depletion of T cells, the infected thymuses eventually regenerate, providing a possible model for studying the process of T cell regeneration. In order to follow this process, we are characterizing T cell repertoire (T cell receptor subtypes) and stem cells in regenerating thymus, using flow cytometry to analyze cells for specific markers. Surprisingly, although these mice remain infected for life, and continually shed virus, they do not generally develop an AIDS-like disease. This contrast may be useful for understanding why, in AIDS, CD<sup>4+</sup> cells are killed but apparently do not regenerate. However, while mice appear normal after recovery from MTLV infection, the thymic damage may have subtle long-term effects, such as autoantibodies.

*Emerging Viruses and Their Evolution.* We are also interested in “emerging viruses” (the origins of “new” viral diseases) and viral evolution, and have been critically examining factors responsible for disease emergence. This interest also led to our developing a PCR system to detect unknown members of the lentivirus subfamily (the group that includes HIV). We have been using this system, and others, to probe lentivirus variation during infection, in order to better understand its pathogenesis and evolution *in vivo*.

**PUBLICATIONS**

- Blankson, J. N., and S. S. Morse. 1994. The CD28/B7 pathway costimulates the response of primary murine T cells to superantigens as well as to conventional antigens. *Cellular Immunology*, 157:306–312.
- Blankson, J. N., D. Y. Loh, and S. S. Morse. 1995. Superantigens and conventional antigens induce different responses in  $\alpha\beta$  T cell receptor transgenic mice. *Immunology*, 85:57–62.
- Gelman, I. H., J. Zhang, E. Hailman, H. Hanafusa, and S. S. Morse. 1992. Identification and evaluation of new primer sets for the detection of lentivirus proviral DNA. *AIDS Research and Human Retroviruses*, 8:1981–1989.
- Morse, S. S. 1993. Examining the origins of emerging viruses. In *Emerging Viruses*. S. S. Morse, editor. Oxford University Press: New York and Oxford.
- Morse, S. S. 1994. Toward an evolutionary biology of viruses. In *The Evolutionary Biology of Viruses*. S. S. Morse, editor. Raven Press: New York.
- Morse, S. S. 1995. Factors in the emergence of infectious diseases. *Emerging Infectious Diseases*, 1:7–15.
- Morse, S. S., and J. E. Valinsky. 1989. Mouse thymic virus (MTLV): A mammalian herpesvirus cytolytic for CD<sup>4+</sup> (L3T<sup>4+</sup>) T lymphocytes. *Journal of Experimental Medicine*, 169:591–596.

### Toby C. Rodman

Our studies are concerned with the repertoire of natural antibodies of the human immune system. The existence of circulating immunoglobulins nonattributable to exogenous antigenic induction has been long recognized, but little definition of their roles as a class or as individual effector molecules has been achieved. We seek to define the primary role of natural antibodies in maintenance of homeostasis and, by virtue of their specific epitopes, their potential secondary role in host defense against infectious invaders.

We have identified the specific epitopes and documented the occurrence of two natural antibodies in all of a large cohort of normal sera. We have determined that their IgM isotypes are present in cord blood at the same level of maturation as that detected in the IgG isotypes of adult blood. Thus, their epitope specificities are innately defined and those antibodies are in their effector state in the neonate. Based upon their ubiquity and identities of their epitopes, we have proposed that those two antibodies represent a class that functions primarily as monitors for aberrant autogenous molecules that may arise in repetitive metabolic processes and the removal of which could prevent errors in critical cellular functions. We postulate that natural antibodies have arisen through mechanisms of survival selection and have become established as an innate repertoire for autosurveillance.

The amino acid organizations of the epitopes for the two natural antibodies identified thus far also support the proposed secondary role, that of defense against an infectious invader, namely HIV. Each of the two epitopes is homologous with a defined functional sequence of HIV Tat protein. Further, the firm documentation of the range of normal titers has enabled us to determine that those antibodies are maintained during the clinically latent period of HIV infection and are depleted as AIDS is approached. Whether and how those antibodies might provide deterrence of HIV pathoprogession is the subject of our continued investigations.

### PUBLICATIONS

Pruslin, F., S. E. To, R. Winston, and T. C. Rodman. 1991. Caveats and suggestions for the ELISA. *Journal of Immunological Methods*, 137:27-33.

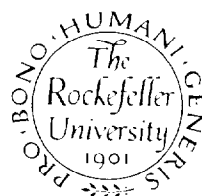
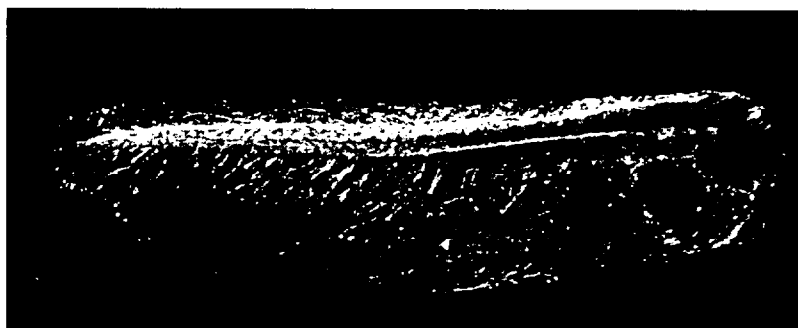
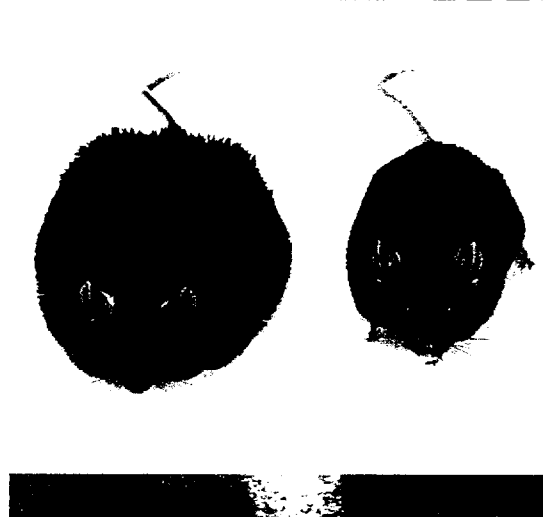
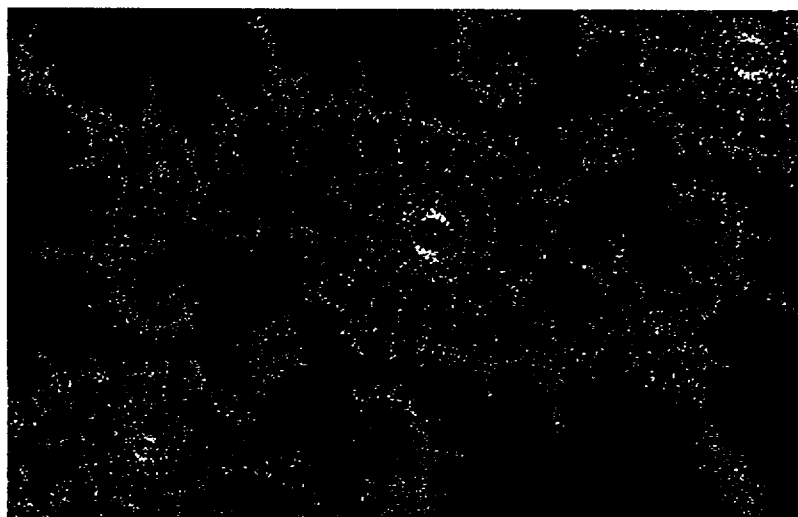
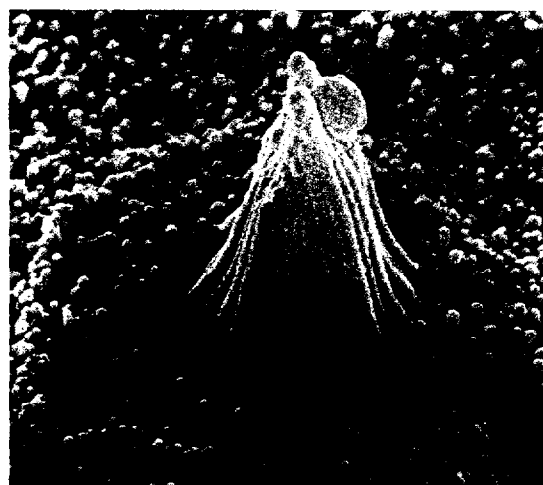
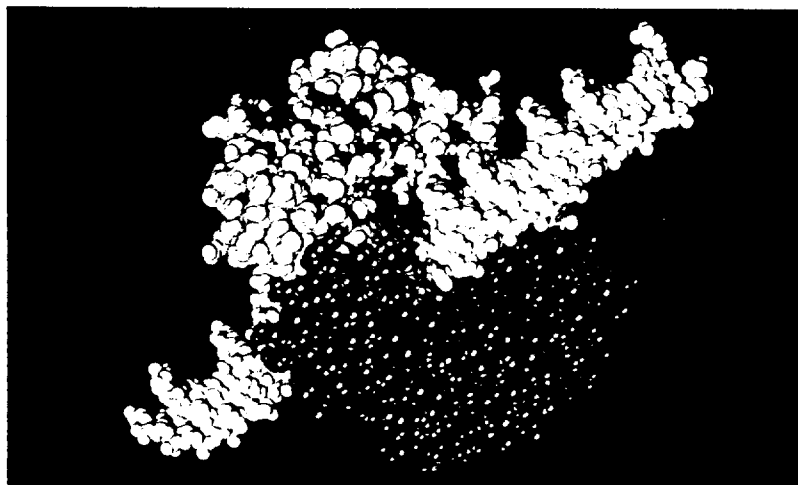
Rodman, T. C., and F. H. Pruslin. 1990. Identification of a low-affinity subset of protamine-reactive antibodies present in normal, deficient in AIDS sera. Implications for HIV latency. *Clinical Immunology and Immunopathology* 57:430-440.

Rodman, T. C., F. H. Pruslin, S. E. To, and R. Winston. 1992. HIV Tat-reactive antibodies present in normal HIV-negative and depleted in HIV-positive sera. Identification of the epitope. *Journal of Experimental Medicine*, 175:1247-1253.

Rodman, T. C., K. M. Manchester, S. E. To, and R. Winston. Antibodies of normal sera with innately defined epitope specificity. Functional implications. In press.

Rodman, T. C., S. E. To, H. Hashish, and K. Manchester. 1993. Epitopes for natural antibodies of HIV-negative and HIV-positive sera are coincident with two key functional sequences of HIV Tat protein. *Proceedings of the National Academy of Sciences USA* 90:7719-7723.

The Rockefeller University  
*1996-1997 Scientific and Educational Programs*



# Joshua Lederberg

## Molecular Genetics and Informatics

Our laboratory focuses on DNA conformation and evolutionary acceleration—namely, how modulation of the secondary and tertiary structure of DNA, and its packaging with protein complexes, influences its vulnerability to chemical alteration. As DNA conformation is known to be a prime actor in the regulation of gene expression (e.g., supercoiling and transcriptional control), this provides a well-founded mechanism for feedback of environmental circumstances and physiological status to the differential mutability of local regions of DNA. A few dozen scattered experimental observations support the thesis, but it has not yet been the subject of systematic critical study. We use small *Escherichia coli* plasmids carrying regulons that can be independently activated by external inducers. These also are associated with change in supercoiling status, which can be regulated *in vivo* and assessed *in vitro*. We are studying how these environmentally controlled variables alter the mutational spectrum consequent to the influence of endogenous metabolism, chemical mutagens and transposons.

### PUBLICATIONS

- Lederberg, J. (1996). Infectious disease—a threat to global health and security. *J. Am. Med. Assoc.* 276:417-418.
- Lederberg, J. (1996). Smaller fleas...*ad infinitum*. Therapeutic bacteriophage redux. *Proc. Natl. Acad. Sci. USA* 93: 3167-3168.
- Lederberg, J. (1996). Options for the future. Symposium on electronic publishing, ICSU/UNESCO, February 22, 1996, Paris, France. *D-Lib Magazine*, May 1996 ISSN 1082-9873.
- Lederberg, J. (1995). Notes on systematic hypothesis generation, and application to disciplined brainstorming. AAAI Symposium, March 27, 1995, Technical Report SS-95-03.

### David S. Thaler

I am a microbial geneticist concerned with the molecular mechanisms, the genetic and formal aspects, and the evolutionary consequences of the ways in which living things change in a hereditary manner. Genetic change is a chemical reaction in which parental genomes are the substrates and new genotypes are the products. We study both the *cis* (chemical and conformational modifications of DNA and RNA) and the *trans* (the enzymes that act on them) components of the reaction.

We showed that, *in vivo*, DNA polymerase can copy short stretches of ribonucleotides as well as other non-canonical templates when they are embedded in normal DNA. This work extends the chemistry that is “alive” because information encoded by the attached bases is accurately inherited. The chemistries studied (RNA, phosphorothioate DNA and RNA, methylphosphonate and 2'-O-methyl RNA) also are used in antisense pharmacology. Our results elevate concerns that antisense molecules may insert information into a cell's DNA. This insertion is a safety concern if the pharmacology is intended only to modulate expression but it might be desirable for gene therapy.

When foreign DNA enters a bacterial cell the major barriers to its informational incorporation are restriction and mismatch repair. Both are subject to genetic and physiological modulation. We discovered and continue to study the role of mismatch repair in regulating the sequence similarity required for recombination. Certain kinds of stress are known to attenuate restriction. We are asking if sublethal doses of antibiotics are such a stress.

Our work is carried out with *E. coli*, *S. typhimurium* and clinical isolates of *Enterococcus* (conjugation of antibiotic (vancomycin) resistance).

### Head of Laboratory

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### Member of the Adjunct Faculty

Susan M. Astrin  
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Toby C. Rodman  
Harriet Zuckerman

### Assistant for Research

Shumo Liu  
James J. Sullivan  
Stephen E. To

## PUBLICATIONS

- Kaplan, P.D., Thaler, D.S., and Libchaber, A. (1996). Overlap extension for construction of computational DNA libraries. Submitted
- Krishnarao, A.S.M., Thaler, D.S., and Goldberg, E.B. (1996). Bacteriophage T4 gp2 interferes with cell viability and with bacteriophage lambda red recombination. Submitted
- Magnasco, M., and Thaler, D.S. (1996). Changing the pace of evolution. *Phys. Lett. A* In press.
- Thaler, D., and Messmer, B. (1996). Genetic intelligence, Evolution of. In: *Encyclopedia of Molecular Biology and Medicine* (Vol. 2). R. Myers, ed. New York: VCH. pp. 407-414.
- Thaler, D.S. (1996). Paradox as path: pattern as map. In: *The Philosophy and History of Molecular Biology: New Perspectives*. S. Sarkar, ed. Netherlands: Kluwer Academic. pp. 233-248.
- Thaler, D.S., Liu, S., and Tomblin, G. (1996). Extending the chemistry that supports genetic information transfer *in vivo*: phosphorothioate DNA, phosphorothioate RNA, 2' O-methyl RNA and methylphosphonate DNA. *Proc. Natl. Acad. Sci. USA* 93:1352-1356.
- Thomason, L.C., Thaler, D.S., Stahl, M.M., and Stahl, F.W. (1996). *In vivo* packaging of bacteriophage lambda monomeric chromosomes. Submitted.
- Thaler, D.S., Tomblin, G., and Zahn, K. (1995). Short patch reverse transcription in *Escherichia coli*. *Genetics* 140:909-915.
- Thaler, D.S. (1994). Adaptive mutation-response. *Science* 265:1995-(1996).
- Thaler, D.S. (1994). Sex is for sisters—intragenomic recombination and homology-dependent mutation as sources of evolutionary variation. *Trends Ecol. Evol.* 9:108-110.
- Thaler, D.S. (1994). The evolution of genetic intelligence. *Science* 264:224-225.
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- Thaler, D.S., Roth, J.R., and Hirschbein, L. (1990). Imprinting as a mechanism for the control of gene expression. In: *The Bacterial Chromosome*. K. Drlica and M. Riley, eds. Washington DC: American Society of Microbiology. pp. 445-456.
- Rayssiguier, C., Thaler, D.S., and Radman, M. (1989). The barrier to recombination between *Escherichia coli* and *Salmonella typhimurium* is disrupted in mismatch-repair mutants. *Nature* 342:396-401.
- Russell, C.B., Thaler, D.S., and Dalquist, F.W. (1989). Chromosomal transformation of *Escherichia coli* recD strains with linearized plasmids. *J. Bacteriol.* 171:2609-2613.
- Thaler, D.S., Sampson, E., Siddiqi, I., et al. (1989). Recombination of bacteriophage lambda in recD mutants of *Escherichia coli*. *Genome* 31(1):53-67.
- Thaler, D.S., and Stahl, F.W. (1988). DNA double-chain breaks in recombination of phage lambda and of yeast. *Annu. Rev. Genet.* 22:169-197.

## Toby C. Rodman

Our studies concern the repertoire of natural antibodies of the human immune system. We seek to define their primary roles that we postulate are related to maintenance of homeostasis and, by virtue of their specific epitopes, their potential secondary roles in host defense against infectious invaders.

For two of the natural antibodies we identified thus far, we applied both trends of study. The amino acid organization of the epitopes of each, with regard to sequence and configuration, supports a primary role as monitor of proteins seeking intracellular entry or permeation of cellular barriers. We determined that those antibodies are present in sera of all of a large cohort of normal humans of broad age span and the IgM isotypes of cord blood are at the same level of maturation as the IgG isotypes of adult blood. That innate occurrence supports the designation of a primary homeostatic role for those antibodies.

With regard to the potential secondary role of those antibodies, the specific epitope for each is embodied in the Tat protein of HIV and each may serve, therefore, to inhibit certain pathoprogenic activities of that viral protein. One epitope embodies the cysteine-rich region of Tat. Immunoreactivity by the natural antibody, therefore, may result in inhibition of cell entry by Tat, a well documented occurrence leading to (a) stimulation of viral replica-

tion or (b) apoptosis of noninfected cells. The Tat-resident epitope for the other antibody is in the arginine-rich region. Long-standing shows that arginine-rich proteins may traverse the blood brain barrier that HIV Tat is capable, *in vitro*, of inducing apoptosis of non-infected neurons. Thus, the prevalent dementia of AIDS may be due to permeation of the blood brain barrier by circulating Tat protein, which, in turn, may be inhibited by the natural antibody specifically reactive with the arginyl-rich sequence of Tat.

Another natural antibody we identified and characterized with regard to its isotype, ubiquitous occurrence and specific reactivity is reactive with lactoferrin, a protein present in all transudates of plasma. The many functions and activities attributed to lactoferrin include cell entry and interaction with DNA. Thus, we postulate again that the natural antibody may serve as a means of surveillance of endocytosis of lactoferrin.

We produced a series of hybridomas, originating from human cord blood B cells, secreting monoclonal antibodies and we ascertained that certain of those monoclonal antibodies are counterparts of the natural antibodies that we identified. Those antibodies will allow us now to probe the postulated actions of circulating natural antibodies in a context more nearly related to that *in vivo* than has hitherto been possible.

#### PUBLICATIONS

Rodman, T.C., Manchester, K.M., To, S.E., Sullivan, J.J., and Winston, R. A primary role of natural antibodies indicated by innate epitope specificity. In press.

Manchester, K., Winston, R., and Rodman, T.C. (1996). Lactoferrin-reactive natural antibodies. New York Academy of Sciences Conference, B Lymphocytes and Autoimmunity.

Rodman, T.C., To, S.E., Hashish, H., and Manchester, K. (1993). Epitopes for natural antibodies of HIV-negative and HIV-positive sera are coincident with two key functional sequences of HIV Tat protein. *Proc. Natl. Acad. Sci. USA* 90: 7719-7723.

Pruslin, F., To, S.E., Winston, R., and Rodman, T.C. (1991). Caveats and suggestions for the ELISA. *J. Immunol. Methods* 137:27-33.

Rodman, T.C., and Pruslin, F.H. 1990. Identification of a low affinity subset of protamine-reactive antibodies present in normal, deficient in AIDS sera. Implications for HIV latency. *Clin. Immunol. Immunopathol.* 57:430-440.

THE ROCKEFELLER UNIVERSITY

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S.E.P

SCIENTIFIC AND EDUCATIONAL PROGRAMS

1997/1998

# Joshua Lederberg

## MOLECULAR GENETICS AND INFORMATICS

### JOSHUA LEDERBERG

Our laboratory focuses on the genetics of evolutionary change in bacteria — namely how DNA structure and metabolism play a part in providing a substrate for evolutionary adaptation. As DNA conformation is already known to be a prime actor in the regulation of gene expression (e.g., supercoiling and transcriptional control), this provides one well-founded mechanism for feedback of environmental circumstances and

physiological status to differential mutability of local regions of DNA. Microbes have also evolved many mechanisms for storing genetic innovation and for cycling through those archives through processes like differential gene expression, phase variation, and transposon hops, as well as plasmid transfer between species. We are seeking experimental tests of how these latter phenomena may also be responsive to environmental signals.

### SELECTED PUBLICATIONS

Lederberg, J. (1996). Infectious disease — a threat to global health and security. *J. Amer. Med. Assn.* 276, 417-418.

Lederberg, J. (1996). Options for the Future. Symposium on electronic publishing, ICSU/UNESCO, February 22, 1996 Paris, France.

### DAVID S. THALER

We are microbial geneticists concerned with the molecular mechanisms, genetics and evolutionary consequences of the ways in which living things change in a hereditary manner. Genetic change is a chemical reaction in which parental genomes are the substrates and new genotypes are the products. We study the *cis* (chemical and conformational modifications of DNA and RNA) and the *trans* (the enzymes that act on them) components of genetic changes.

We have shown that short stretches of ribonucleotides as well as other noncanonical templates are genetically potent *in vivo* when they are embedded in normal DNA. This work extends the chemistry that is “alive” because information encoded by the attached bases is accurately inherited. Chemistry is only one component of the genetic, physiological and physical barriers to the incorporation of potentially hereditary information. Other barriers include the amount of divergence between sequences,

restriction and physical separation. With regard to physical separation, we are exploring cell fusion as a way to bring two bacterial genomes into the same cytoplasm to give the genomes a new sort of opportunity to interact.

In collaboration with Toby Rodman we are using phage display libraries to characterize epitopes recognized by natural antibodies. The goal of this project is to understand the repertoire of shapes recognized by the neonate immune system.

Genetic change is an informational as well as a chemical process. In collaboration with members of the university's Center for Studies in Physics and Biology we have worked on computing with DNA, i.e., performing algorithmic computations by molecular-genetic methods. With Assistant Professor Marcelo Magansco of the center we consider the evolutionary consequences of the fact that organisms inherit the genetically determined algorithms by which evolutionary variation is generated.

### HEAD OF LABORATORY

Professor Emeritus  
Joshua Lederberg  
President Emeritus  
Raymond and Beverly  
Sackler Foundation Scholar

### ASSOCIATE PROFESSOR

David S. Thaler

### ASSISTANT PROFESSOR

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### MEMBERS OF THE ADJUNCT FACULTY

Robert K. Merton  
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Toby C. Rodman  
Harriet Zuckerman

### SELECTED PUBLICATIONS

Kaplan, P.D., Ouyang, Q., Thaler, D.S., and Libchaber, A. (1997). Parallel overlap extension for construction of computational CNA libraries. *J. Theor. Biol.* In press.

Krishnarao, A.S.M., Thaler, D.S., and Goldberg, E.B. (1997). Bacteriophage T4 gp2 interferes with cell viability and with bacteriophage lambda red recombination. *J. Bacteriol.* In press.

Moxon, E.R., and Thaler, D.S. (1997). The tinkerer's evolving toolbox. *Nature* 387, 659-662.

Thomason, L.C., Thaler, D.S., Stahl, M.M., and Stahl, F.W. (1997). *In vivo* packaging of bacteriophage lambda monomeric chromosomes. *J. Mol. Biol.* 276, 1-13.

Magnasco, M., and Thaler, D.S. (1996). Changing the pace of evolution. *Phys. Lett. A* 221, 287-292.

Thaler, D.S. (1996). Paradox as path: Pattern as map. In *The Philosophy and History of Molecular Biology: New Perspectives*. Sarkar, S., ed. (Netherlands: Kluwer Academic), pp. 233-248.

MOLECULAR, CELL AND DEVELOPMENTAL BIOLOGY/IMMUNOLOGY AND MICROBIOLOGY

Thaler, D.S., Liu, S., and Tomblin, G. (1996). Extending the chemistry that supports genetic information transfer *in vivo*: phosphorothioate DNA, phosphorothioate RNA, 2' O-methyl RNA and methylphosphonate DNA. *Proc. Natl. Acad. Sci. USA* 93, 1352-1356.

Thaler, D.S., Messmer, B.T. (1996). Genetic Intelligence, Evolution of. In *Encyclopedia of Molecular Biology and Medicine*, vol. 2, Myers, R., ed. (New York: VCH). pp. 407-414.

Thaler, D.S., Tomblin, G., and Zahn, K. (1995). Short patch reverse transcription in *Escherichia coli*. *Genetics* 140, 909-915.

Thaler, D.S. (1994). Adaptive mutation-response. *Science* 265, 1994-1996.

Thaler, D.S. (1994). The evolution of genetic intelligence. *Science* 264, 224-225.

Thaler, D.S. (1994). Sex is for sisters—intragenomic recombination and homology-dependent mutation as sources of evolutionary variation. *Trends Ecol. Evol.* 9, 108-110.

Le Derout, J., Thaler, D.S., Guillen, N., and Hirschbein, L. (1992). The spoOA gene is implicated in the maintenance of non-complementing diploids in *Bacillus subtilis*. *Molec. Microbiol.* 6, 1495-1505.

Thaler, D.S., and Noordewier, M.O. (1992). MEPS parameters and graph analysis for the use of recombination to construct ordered sets of overlapping clones. *Genomics* 13, 1065-1074.

Thaler, D.S., Roth, J.R., and Hirschbein, L. (1990). Imprinting as a mechanism for the control of gene expression. In *The Bacterial Chromosome*. Drlica, K., and M. Riley, eds. (Washington, D.C.: American Society of Microbiology), pp. 445-456.

Rayssiguier, C., Thaler, D.S., and Radman, M. (1989). The barrier to recombination between *Escherichia coli* and *Salmonella typhimurium* is disrupted in mismatch-repair mutants. *Nature* 342, 396-401.

Russell, C.B., Thaler, D.S., and Dalquist, F.W. (1989). Chromosomal transformation of *Escherichia coli* recD strains with linearized plasmids. *J. Bacteriol.* 171, 2609-2613.

Thaler, D.S., Sampson, E., Siddiqi, I., et al. (1989). Recombination of bacteriophage lambda in recD mutants of *Escherichia coli*. *Genome* 31, 53-67.

Thaler, D.S., and Stahl, F.W. (1988). DNA double-chain breaks in recombination of phage lambda and of yeast. *Ann. Rev. Genet.* 22, 169-197.

## SRINIVAS SASTRY

We study mechanisms of transcriptional initiation and elongation. Our goal is to understand the conformational transitions in the RNA polymerase molecule as it moves down the DNA template. We are mapping the DNA and RNA contacting amino acid side chains in the RNA polymerase using photochemical cross-linking approaches. Transcriptional initiation and elongation complexes of *Escherichia coli* and phage T7 RNA polymerases are prepared by arresting the polymerase at specific sites on the DNA template and nascent RNA are induced by high intensity UV light of specific wavelengths, and a site-specific photochemical probe on the DNA template or in the RNA. Highly stable transcription initiation and elongation complexes are purified by high performance liquid chromatography (HPLC). The molecular contacts between the amino acid side chains in the RNA polymerase and the DNA and RNA bases are mapped by a variety of techniques that include

proteolytic degradation of the polymerase, mass spectrometry, amino acid composition analysis and peptide sequence analysis.

### The Repair of DNA-Protein Cross-Links.

The formation and "repair" of protein-DNA cross-links is a poorly understood phenomenon. My hypothesis is that the protein-DNA cross-link is a major source of intracellular DNA damage, and that protein-DNA cross-links are formed at one to two orders of magnitude greater than other types of DNA damage, such as interstrand DNA cross-links (about eight per cell per day). To understand the mechanisms of "repair" of DNA-protein cross-links we are constructing UV-mediated zero-length and chemical adduct-mediated protein-DNA cross-links. "Frozen" topoisomerase-DNA intermediates, which may occur naturally under certain conditions, are also being used as model substrates for the "repair" assays. As a first step, we are developing assays using human cell-free extracts.

## SELECTED PUBLICATIONS

Sastry, S.S. (1997). Isolation and partial characterization of a novel psoralen-tyrosine photoconjugate from a photoreaction of psoralen with a natural protein. *Photochem. Photobiol.* 65, 937-944.

Sastry, S.S., and Ross, B.M. (1997). Nuclease activity of T7 RNA polymerase and the heterogeneity of transcription elongation complexes. *J. Biol. Chem.* 272, 8644-8652.

Sastry, S.S., and Ross, B.M. (1997). Probing the mechanism of T7 RNA polymerase transcription initiation using covalent

photochemical conjugation of psoralen to a promoter. *Biochemistry* 36, 3133-3144.

Sastry, S.S., Ross, B.M., and Parraga, A. (1997). Crosslinking of DNA-binding proteins to DNA with psoralen and psoralen furan side mono adducts: comparison of action spectra with DNA-DNA crosslinking. *J. Biol. Chem.* 272, 3715-3723.

Sastry, S.S. (1996). Identification of the template-binding cleft of T7 RNA polymerase as the site for promoter binding by photochemical crosslinking with psoralen. *Biochemistry* 35, 13519-13530.

Sastry, S.S., and Ross, B.M. (1996). A direct real-time spectroscopic investigation of the mechanism of open complex formation by T7 RNA polymerase. *Biochemistry* 35, 15715-15725.

Sastry, S.S., and Hoffman, P.L. (1995). The influence of RNA and DNA template structure during transcript elongation by RNA polymerases. *Biochem. Biophys. Res. Comm.* 211, 106-114.

Spielmann, H.P., Dwyer, T.J., Sastry, S.S., Hearst, J.E., and Wemmer, D.E. (1995). DNA conformational reorganization upon conversion of a psoralen furan-side monoadduct to an interstrand cross-link: implications for DNA repair. *Proc. Natl. Acad. Sci. USA* 92, 2345-2349.

Sastry, S.S., Spielmann, H.P., Hoang, Q.S., Phillips, A.M., Sancar, A., and Hearst, J.E. (1993). Laser-induced protein-DNA cross-links via psoralen furanside monoadducts. *Biochemistry* 32, 5526-5538.

Sastry, S.S., Spielmann, H.P., and Hearst, J.E. (1992). Psoralens and their application to the study of some molecular biological processes. *Adv. Enzymol.* 66, 85-148.

## TOBY C. RODMAN

The effector molecules of the immune system include a repertoire of circulating immunoglobulins nonattributable to exogenous antigenic induction, variously designated "autoantibodies" or "natural antibodies." The latter term is customarily applied to those antibodies for which beneficial function is demonstrated or predicated, e.g., xenogeneic rejection, support of homeostasis or host factor of defense against infectious invaders. Since the effective performance of each of those roles is dependent upon specific recognition by the antibody for the molecular entity or epitope to which it binds, we have sought to establish methodology and criteria for epitope definition and for determination of state of maturation of natural antibodies throughout the life span. Thus far, by application of those methods, we have identified three natural human antibodies. The successful identification of their epitope specificities, and characterization of each as innately differentiated, support the potential usefulness of this trend of study in: (a) identification of natural host factors of homeostasis and defense and (b) probing their evolutionary development.

We identified the epitope for a specific natural IgM antibody as a cryptic sequence of lactoferrin, a ubiquitous glycoprotein found in body fluids and on certain cell surfaces. Lactoferrin has been shown to be capable of cell entry and DNA binding. The presence of the natural antibody, therefore, may provide a providential control of those capabilities of lactoferrin in physiological instances when such intervention may be homeostatically expedient. The innate occurrence of that natural antibody is shown by our production of a hybridoma, derived from a human cord blood B cell, secreting the Mab counterpart of the circulating natural anti-lactoferrin antibody.

For two other natural antibodies we established life-span occurrence in a large cohort

of normal humans and maintenance of steady titer by individuals that, in turn, may be a mechanism for meeting homeostatic demand. The determined epitope specificity of each of those antibodies is compatible with a primary role as monitor of intracellular traffic. Significant secondary roles of those antibodies as agents of defense against an infectious invader are supported by identification of the specific epitope of each as a defined functional sequence of the Tat protein of HIV. The role(s) of the Tat protein in the pathoprogession of HIV infection include participation in cellular entry of the virus and cell destruction by free circulating Tat protein. The epitope for one of the natural antibodies embodies the cysteine-rich region of Tat, which has been shown to participate in cell entry. That natural antibody, therefore, may play a role in restriction of Tat-mediated T4 cell entry by HIV. For the second Tat-reactive natural antibody, the Tat-resident epitope encompasses the arginine-rich sequence. We produced a hybridoma that secretes an antibody homologous with the arginyl-sequence-reactive natural antibody. The derivation of that hybridoma from a human cord blood B cell verifies the innate character of that natural antibody. There is long-standing evidence that arginine-rich proteins may traverse the blood brain barrier and recent evidence that HIV Tat is capable, *in vitro*, of inducing apoptosis of non-infected neurons. Therefore, in addition to its general pathologic effects, circulating Tat protein may, through its arginyl sequence, permeate the blood brain barrier and instigate the dementia of AIDS. In turn, that progression may be retarded by the circulating arginyl-sequence-reactive natural antibody. The data of our current studies show that the Mab counterpart of the natural antibody is capable of inhibiting Tat activity *in vitro*.

SELECTED PUBLICATIONS

- Rodman, T.C., To, S.T., Sullivan, J., and Winston, R. (1997). Innate natural antibodies. Primary role indicated by specific epitopes. *Hum. Immunol.* In press.
- Rodman, T.C., Winston, R., Sullivan, J., Yan, X-J., and Chiorazzi, N. (1997). An innate natural antibody is reactive with a cryptic sequence of lactoferrin exposed on sperm head surface. *Proc. Soc. Exp. Biol. Med.* In press.
- Manchester, K., Winston, R., and Rodman, T.C. (1996). Lactoferrin-reactive natural antibodies. *Ann. N.Y. Acad. Sci.* 815, 475-477.
- Rodman, T.C., To, S.E., Hashish, H., and Manchester, K. (1993). Epitopes for natural antibodies of HIV-negative and HIV-positive sera coincident with two key functional sequences of HIV Tat protein. *Proc. Natl. Acad. Sci. USA* 90, 7719-7723.
- Rodman, T.C., Pruslin, F.H., To, S.E., and Winston, R. (1992). Human immunodeficiency virus (HIV) Tat-reactive antibodies present in normal HIV-negative sera and depleted in HIV-positive sera. Identification of the epitope. *J. Exp. Med.* 175, 1247-1253.
- Pruslin, F., To, S.E., Winston, R., and Rodman, T.C. (1991). Caveats and suggestions for the ELISA. *J. Immunol. Methods* 137, 27-33.
- Rodman, T.C., and Pruslin, F.H. (1990). Identification of a low affinity subset of protamine-reactive antibodies present in normal, deficient in AIDS sera. Implications for HIV latency. *Clin. Immunol. Immunopathol.* 57, 430-440.

# The Rockefeller University

SCIENTIFIC AND EDUCATIONAL  
PROGRAMS 1998-1999



# Joshua Lederberg

## MOLECULAR GENETICS AND INFORMATICS

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### ASSOCIATE PROFESSOR

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### ASSISTANT PROFESSOR

Srinivas Sastry

### GRADUATE FELLOWS

Bradley Messmer  
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### MEMBERS OF THE ADJUNCT FACULTY

Robert K. Merton  
Stephen S. Morse  
Harriet Zuckerman

### Joshua Lederberg

Our laboratory is focused on the genetics of evolutionary change in bacteria—namely how DNA structure and metabolism play a part in providing a substrate for evolutionary adaptation. In addition, we are seeking new modes of cell fusion in *E. coli*, besides the systems of conjugation, plasmid transfer, phage-mediated transduction and DNA-mediated transforma-

tion, that have been the methodological basis of genetic engineering.

On the theoretical and policy-oriented side, I have been very much concerned with analyzing the looming threat of emerging and re-emerging infection, and developing the global public health systems that might help defend us.

### SELECTED PUBLICATIONS

Lederberg, J. (1997). *Infectious disease as an evolutionary paradigm*. *Emerging Infect. Dis.* 3, 417-423.

Lederberg, J. (1997). *Some early stirrings (1950 ff) of concern about environmental mutagens*. *Env. Mol. Mutagenesis*. 30, 3-10.

### David S. Thaler

Genotypes change via mutation and recombination. These are chemical reactions in which parental genomes are the substrates and new genotypes are the products. We have found that short stretches of ribonucleotides and other noncanonical templates (phosphorothioate, 2'-O-methyl RNA and methylphosphonate DNA) are genetically potent when they are embedded in normal DNA. We call the ribonucleotide copying process Short Patch Reverse Transcription (SPRT). This work extends the chemistry that is alive because information encoded by the attached bases is inherited in subsequent generations.

The molecular mechanisms for genetic change are also affected by the amount of divergence between sequences (regulated by the mismatch correction system), DNA restriction and physical separation. We study each of these systems. With regard to physical separation, we are exploring cell fusion as a way to bring two bacterial genomes into the same cytoplasm to give the genomes a new way to interact. Conjugation can also transfer DNA between different species of bacteria. New mechanisms by which microbial diversity is generated are fascinating from a theoretical perspective; from the perspective of emerging infections they are

disturbing. A particular case of interest is a clinical isolate of *Enterococcus faecium* that conjugates a chromosomally-located vancomycin resistance cluster into another species of *Enterococcus*. In this system, large and variable segments of the donor chromosome (90-300 kb) are transferred during conjugation and alters many aspects of the recipient in addition to conferring vancomycin resistance. In an important sense each transconjugant is a new species.

Evolving pathogens may be considered the offense and the evolving immune system the defense. We study diversity of the defense as well as the offense. We are using phage displayed libraries of peptides to characterize the epitopes recognized by antibodies. A goal of one project is to determine the repertoire of shapes recognized by the neonate immune system.

Genetic change and evolution are informational as well as chemical processes. In collaboration with members of the Rockefeller University Center for the Studies in Physics and Biology we have worked on computing with DNA. Assistant Professor Marcelo Magansco of the Center and I collaborate to study the informational and evolutionary consequences of genetic mechanisms.

## SELECTED PUBLICATIONS

- Kaplan, P.D., Thaler, D.S., and Libchaber, A. (1998). Parallel overlap assembly of paths through a directed graph. *Proceedings of the 3rd DIMACS workshop on DNA based computers*. American Mathematical Soc. In press.
- Krishnarao, A.S.M., Thaler D.S., and Goldberg, E.B. (1998). Bacteriophage T4 gp2 interferes with cell viability and with bacteriophage lambda red recombination. *J. Bacteriol.* In press.
- Kaplan, P.D., Ouyang, Q., Thaler, D.S., and Libchaber, A. (1997). Parallel overlap extension for construction of computational DNA libraries. *J. Theor. Biol.* 188, 333-341.
- Moxon, E.R., and Thaler D.S. (1997). The tinkerer's evolving toolbox. *Nature* 387, 659-662.
- Thomason, L.C., Thaler, D.S., Stahl, M.M., and Stahl, F.W. (1997). In vivo packaging of bacteriophage lambda monomeric chromosomes. *J. Mol. Biol.* 267, 1-13.
- Magnasco, M., and Thaler, D.S. (1996). Changing the pace of evolution. *Phys. Lett. A* 221, 287-292.
- Thaler, D.S. (1996). Paradox as path: pattern as map. In *The Philosophy and History of Molecular Biology: New Perspectives*. S. Sarkar, ed. (Netherlands: Kluwer Academic), 233-248.
- Thaler, D.S., Liu, S., and Tomblin, G. (1996). Extending the chemistry that supports genetic information transfer in vivo: phosphorothioate DNA, phosphorothioate RNA, 2'-O-methyl RNA and methylphosphonate DNA. *Proc. Natl. Acad. Sci. USA* 93, 1352-1356.
- Thaler, D.S., and Messmer, B.T. (1996). Genetic intelligence, evolution of. In: *Encyclopedia of Molecular Biology and Medicine*. vol. 2, R. Myers, ed. (New York: VCH), pp. 407-414.
- Thaler, D.S., Tomblin, G., and Zahn K. (1995). Short patch reverse transcription in *Escherichia coli*. *Genetics* 140, 909-915.
- Thaler, D.S. (1994). The evolution of genetic intelligence. *Science* 264, 224-225.
- Thaler, D.S. (1994). Sex is for sisters-intragenomic recombination and homology-dependent mutation as sources of evolutionary variation. *Trends Ecol. Evol.* 9, 108-110.
- Rayssiguier, C., Thaler, D.S., and Radman, M. (1989). The barrier to recombination between *Escherichia coli* and *Salmonella typhimurium* is disrupted in mismatch-repair mutants. *Nature* 342, 396-401.

## Srinivas Sastry

Immunization with heat shock protein (HSPs) preparations isolated from cancer cells or virus-infected cells elicits protective anti-tumor or anti-viral cellular immunity. The basis for this paradigm is that peptides that are generated in cells from which the HSPs are isolated, are bound noncovalently to HSPs and are responsible for the antigenicity. In collaboration with Antigenics Corporation and Dr. Pramod Srivastava's Laboratory at the University of Connecticut, we are studying the mechanism of association of peptides with a mouse model antigenic system viz., gp96. Our goal is to understand the kinetics and dynamics of the interaction of immunogenic peptides to the chaperone gp96. Is there selectivity/specificity in the binding of peptides to gp96? How are the peptides bound in the interior cavity (and/or the exterior surface) of gp96? What are the residues in gp96 that interact with peptides? What are the conformational transitions that occur in gp96 when peptides are loaded? We are using highly sensitive fluorescence, CD spectroscopy and cross-linking techniques to answer these and related questions. This work attempts to relate the mechanisms of peptide-binding to HSP chaperones and their antigen presentation mechanisms to MHC class I molecules in the generation cellular immunity.

**Novel DNA Repair Mechanisms.** The mech-

anisms of formation and repair of protein-DNA cross-links is our research focus. My hypothesis is that the protein-DNA cross-link is a major source of intracellular DNA damage. Protein-DNA cross-links may be formed in vivo at a much greater rate than other types of DNA damage such as inter-strand DNA cross-links (about 8 per cell per day). Frozen topoisomerase-DNA cross-links, which occur in patients undergoing chemotherapy with topoisomerase poisons, are an important source of DNA damage. To understand DNA repair mechanisms in these systems, we are constructing models of UV-mediated zero-length cross-links, chemical-adduct cross-links and topo-DNA cross-links. Using these models, we have discovered new enzymatic pathways that process DNA-protein cross-links by human cell-free extracts.

#### Transcription Initiation and Elongation.

Our goal is to understand the conformational transitions in the RNA polymerase molecule as it moves down the DNA template. We are mapping the DNA and RNA contacting amino acid side chains in the RNA polymerase using photochemical cross-linking approaches.

Transcriptional initiation and elongation complexes of phage T7 RNA polymerases are prepared by arresting the polymerase at specific sites on the DNA templates. Specific cross-link-

ing of the arrested RNA polymerase to the DNA template and nascent RNA are induced by high intensity UV light and a site-specific photochemical probe attached to the DNA template or the RNA. Highly stable transcription initiation and elongation complexes are purified by high-performance liquid chromatography

(HPLC) and the molecular contacts between the amino acid side chains in the RNA polymerase and the DNA and RNA bases are mapped by a variety of techniques that include proteolytic degradation of the polymerase, mass spectrometry, amino acid composition analysis and peptide sequence analysis.

#### SELECTED PUBLICATIONS

Sastry, S.S., and Ross, B.M. (1998). Mechanisms for the processing of a frozen topoisomerase-DNA conjugate by human cell-free extracts. *J. Biol. Chem.* 273, 9942-9950.

Sastry, S.S., and Ross, B.M. (1998). RNA binding site in T7 RNA polymerase. *Proc. Natl. Acad. Sci. USA*. In press.

Sastry, S.S. (1997). Isolation and partial characterization of a novel psoralen-tyrosine photoconjugate from a photoreaction of psoralen with a natural protein. *Photochem. Photobiol.* 65, 937-944.

Sastry, S.S., and Ross, B.M. (1997). Nuclease activity of T7 RNA polymerase and the heterogeneity of transcription elongation complexes. *J. Biol. Chem.* 272, 8644-8652.

Sastry, S.S., and Ross, B.M. (1997). Probing the mechanism of T7 RNA polymerase transcription initiation using covalent photochemical conjugation of psoralen to a promoter. *Biochemistry* 36, 3133-3144.

Sastry, S.S., Ross, B.M., and Parraga, A. (1997). Crosslinking of DNA-binding proteins to DNA with psoralen and psoralen furan side mono adducts: comparison of action spectra with DNA-DNA crosslinking. *J. Biol. Chem.* 272, 3715-3723.

Sastry, S.S. (1996). Identification of the template-binding cleft of T7 RNA polymerase as the site for promoter binding by photochemical cross-linking with psoralen. *Biochemistry* 35, 13519-13530.

Sastry, S.S., and Ross, B.M. (1996). A direct real-time spectroscopic investigation of the mechanism of open complex formation by T7 RNA polymerase. *Biochemistry* 35, 15715-15725.

Sastry, S.S., and Hoffman P.L. (1995). The influence of RNA and DNA template structure during transcript elongation by RNA polymerases. *Biochem. Biophys. Res. Comm.* 211, 106-114.

Spielmann, H.P., Dwyer, T.J., Sastry, S.S., Hearst, J.E., and Wemmer, D.E. (1995). DNA conformational reorganization upon conversion of a psoralen furan-side monoadduct to an interstrand cross-link: Implications for DNA repair. *Proc. Natl. Acad. Sci. USA* 92, 2345-2349.

Sastry, S.S., Spielmann, H.P., Hoang, Q.S., Phillips, A.M., Sancar, A., and Hearst, J.E. (1993). Laser-induced protein-DNA cross-links via Psoralen Furanside monoadducts. *Biochemistry* 32, 5526-5538.

Sastry, S.S., Spielmann, H.P., and Hearst, J.E. (1992). Psoralens and their application to the study of some molecular biological processes. *Adv. Enzymol.* 66, 85-148.